CLAIM AMENDMENTS

Please cancel claims 15, 38, and 47, without prejudice or disclaimer, as being directed to subject matter that has been withdrawn from consideration.

Please amend the claims as follows:

1. (currently amended) A method for purifying poly(A) mRNA from a sample in a manner that reduces rRNA carryover comprising:

a) incubating a composition comprising:

i) the sample, wherein the sample includes poly(A) mRNA;

ii) a poly(dT) or poly(U) nucleic acid molecule; and

iii) ~~an isostabilizing agent, wherein the isostabilizing agent is~~ tetramethylammonium chloride (TMAC) or tetraethylammonium chloride (TEAC),

under conditions allowing poly(A) mRNA to hybridize with the poly(dT) or poly(U) nucleic acid molecule and inhibiting partial hybridization of the poly(A) mRNA to any rRNA that may be present in the sample; and

b) isolating the poly(dT) or poly(U) nucleic acid molecule and the hybridized poly(A) mRNA[[.]];

wherein rRNA carryover is reduced.

2. (currently amended) The method of claim 1, wherein the final concentration of the ~~isostabilizing agent~~ TMAC and/or TEAC in the composition is between about 1.0 M and about 3.0 M.

3. (currently amended) The method of claim 2, wherein the final concentration of the ~~isostabilizing agent~~ TMAC and/or TEAC in the composition is between about 1.2 M and about 2.4 M.

4. (currently amended) The method of claim 3, wherein the final concentration of the ~~isostabilizing agent~~ TMAC and/or TEAC in the composition is between about 1.5 M and about 2.0 M.

5. (currently amended) The method of claim 1, wherein the ~~isostabilizing agent~~ TMAC or TEAC is provided to the composition in a hybridization solution.

6. (original) The method of claim 1, wherein the composition further comprises CHAPS in a final concentration between about 0.5% and about 2.0%.

7. (original) The method of claim 1, wherein the composition further comprises Triton X-100.

8. (original) The method of claim 7, wherein the concentration of Triton X-100 in the composition is between about 0.01% and about 0.1%.

9. (original) The method of claim 5, wherein the hybridization solution further comprises Triton X-100.

10. (original) The method of claim 1, further comprising heating the composition at a temperature between about 70°C and about 90°C prior to incubation under hybridization conditions.

11. (original) The method of claim 1, wherein the hybridization conditions comprise incubating the composition between about 15°C and 50°C for at least 10 minutes to 48 hours.

12. (original) The method of claim 11, wherein the incubation time is at least 4 hours.

13. (currently amended) The method of claim 1, further comprising washing the poly(dT) or poly(U) nucleic acid molecule and the hybridized poly(A) mRNA in wash solution comprising ~~an isostabilizing agent~~ TMAC or TEAC.

14. (previously presented) The method of claim 13, wherein the poly(dT) or poly(U) nucleic acid molecule and the hybridized poly(A) mRNA are washed more than once.

15. (cancelled)

16. (currently amended) The method of claim 14, wherein the concentration of the ~~isostabilizing agent~~ TMAC and/or TEAC in the wash solution is between about 0.05 M and about 3.0 M.

17. (currently amended) The method of claim 14, wherein the poly(dT) or poly(U) nucleic acid molecule and the hybridized poly(A) mRNA are washed at least once in a wash solution with ~~an isostabilizing agent~~ a TMAC and/or TEAC concentration greater than about 1.2 M and at least once in a wash solution with a TMAC and/or TEAC concentration of less than about 0.5 M.

18. (original) The method of claim 1, wherein the poly(dT) or poly(U) nucleic acid molecule is linked to a non-reacting structure.

19. (original) The method of claim 18, wherein the non-reacting structure is cellulose.

20. (previously presented) The method of claim 18, further comprising isolating the non-reacting structure linked to the oligonucleotide that is hybridized to poly(A) mRNA.

21. (original) The method of claim 20, further comprising washing the non-reacting structure.

22. (original) The method of claim 18, wherein the non-reacting structure is a bead.

23. (original) The method of claim 22, wherein the bead is magnetic.

24. (previously presented) The method of claim 23, wherein the poly(dT) or poly(U) nucleic acid molecule and the hybridized poly(A) mRNA are isolated from the sample with a magnet.

25. (original) The method of claim 20, wherein the non-reacting structure is isolated from the sample by centrifugation or filtration.

26. (previously presented) The method of claim 18, further comprising eluting the poly(A) mRNA from the non-reacting structure with an eluting solution of low ionic strength.

27. (original) The method of claim 26, wherein the eluting solution comprises sodium citrate.

28. (original) The method of claim 1, wherein the poly(dT) or poly(U) nucleic acid molecule is biotinylated.

29. (previously presented) The method of claim 28, further comprising

c) incubating the biotinylated oligonucleotide and the sample with avidin or streptavidin linked to a non-reacting structure; and

d) eluting the poly(A) mRNA from the non-reacting structure with an eluting solution.

30. (original) The method of claim 1, wherein the sample or the hybridization solution does not contain guanidinium.

31. (currently amended) A method for purifying poly(A) mRNA from a sample <u>in a manner that reduces rRNA carryover</u> comprising:

a) incubating the sample with a poly(dT) oligonucleotide connected to a non-reacting structure and a hybridization solution comprising tetramethylammonium under conditions allowing poly(A) mRNA to hybridize with the oligonucleotide;

b) isolating the oligonucleotide with the hybridized poly(A) mRNA away from the sample; and

c) washing the oligonucleotide with a wash solution comprising a salt[[.]];

<u>wherein rRNA carryover is reduced.</u>

32. (original) The method of claim 31, wherein the non-reacting structure is cellulose.

33. (original) The method of claim 31, wherein the oligonucleotide is biotinylated.

34. (previously presented) The method of claim 33, further comprising

c) incubating the biotinylated oligonucleotide and the sample with avidin or streptavidin linked to a non-reacting structure; and

d) eluting the poly(A) mRNA from the non-reacting structure with an eluting solution.

35. (previously presented) The method of claim 34, further comprising isolating the non-reacting structure linked to the oligonucleotide hybridized to poly(A) mRNA by centrifugation or filtration.

36. (previously presented) The method of claim 31, further comprising eluting the poly(A) mRNA from the non-reacting structure with an eluting solution with low ionic strength.

37. (currently amended) A kit, in a suitable container means, comprising:

a) a poly(dT) oligonucleotide linked to a non-reacting structure; and

b) binding solution comprising ~~an isostabilizing agent~~ TMAC or TEAC.

38. (cancelled)

39. (currently amended) The kit of claim 38, wherein the concentration of TMAC and/or TEAC in the binding solution is between about 1.0 M and about 5.0 M.

40. (currently amended) The kit of claim 39, wherein the concentration of TMAC and/or TEAC in the binding solution is about 4.0 M.

41. (currently amended) The kit of claim 39, wherein the concentration of TMAC and/or TEAC in the binding solution is about 2.0 M.

42. (original) The kit of claim 37, wherein the binding solution further comprises at least one detergent.

43. (original) The kit of claim 42, wherein the detergent is Triton X-100 or CHAPS, or a combination of Triton X-100 and CHAPS.

44. (original) The kit of claim 43, wherein the concentration of the detergent in the binding solution is between about 0.001% to about 1.0%.

45. (original) The kit of claim 37, further comprising a detergent in a concentration of between about 0.01% and 0.1%.

46. (currently amended) The kit of claim 37, further comprising a wash solution comprising ~~an isostabilizing agent~~ TMAC or TEAC.

47. (cancelled)

48. (currently amended) The kit of claim 46, wherein the concentration of TMAC and/or TEAC in the wash solution is between about 0.1 M and about 2.0 M.

49. (currently amended) The kit of claim 48, wherein the concentration of TMAC and/or TEAC in the wash solution is about 2.0 M.

50. (original) The kit of claim 37, further comprising an elution solution of low ionic strength comprising a chelating salt.

51. (original) The kit of claim 50, wherein the salt in the elution solution is sodium citrate or EDTA-2Na.

52. (original) The kit of claim 50, wherein the concentration of the salt in the elution solution is between about 0.1 mM and about 100 mM.

53. (original) The kit of claim 37, wherein the oligonucleotide is biotinylated.

54. (original) The kit of claim 53, wherein the non-reacting structure is a streptavidin or avidin matrix.

55. (original) The kit of claim 37, wherein the non-reacting structure is cellulose.

56. (original) The kit of claim 37, wherein the non-reacting structure is a bead.

57. (original) The kit of claim 56, wherein the bead is magnetic.

58. (original) The kit of claim 57, further comprising a magnetic stand.

59. (original) The kit of claim 37, further comprising a filtration device.

60. (original) A kit, in suitable container means, comprising:

a) a poly(dT) oligonucleotide linked to cellulose;

b) hybridization solution comprising tetramethylammonium (TMAC) in a concentration of between about 1.2 M and about 4 M and Triton X-100 in a concentration of between about 0.03% and about 0.1%;

c) a first wash solution comprising TMAC in a concentration of about 2 M;

d) a second wash solution comprising TMAC in a concentration of about 0.4 M; and

e) elution solution having a total ionic strength of less than 0.01.

61. (previously presented) The method of claim 1, wherein the isostabilizing agent is TMAC, and wherein the nucleic acid molecule is poly(dT) and is linked to a non-reacting structure, and further comprising c) washing the poly(dT) nucleic acid molecule with a wash solution comprising a salt.

RESPONSE

A. Status of the Claims

Claims 1-60 were initially filed. Claims 1, 13, 14, 17, 20, 24, 26, 29, 31, and 34-36 were amended and claim 61 was added in an Amendment filed concurrently with the application. Claims 15, 38, and 47 are canceled herein. Therefore, claims 1-14, 16-37, 39-46, and 48-61 are currently pending and presented herein for reconsideration.

Claims 1-5, 13, 16, 17, 31, 37, 39-41, 46, 48 and 49 have been amended to clarify the invention and provide the proper antecedent basis for the claims. Support for the amendments may be found throughout the specification, for example, in Example 1, Example 2, and Example 4. No new matter has been added. These amendments were previously filed on October 29, 2003, but were not entered.

B. Rejections Under 35 U.S.C. § 103(a)

The Action alleges that claims 1-5, 10-18, 20-28, 30-33, 36-38, 41, 42, 45-50, 53-59, and 61 are unpatentable over Kearney *et al.* in view of Aviv *et al.* Applicant respectfully traverses.

1. Method Claims 1-5, 10-18, 20-28, 30-33, 36 Are Allowable Over Kearney *et al.* in View of Aviv *et al.*

Claims 1-36 are directed to methods, as exemplified by independent claims 1 and 31. Present claim 1 is directed to:

> A method for purifying poly(A) mRNA from a sample in a manner that reduces rRNA carryover comprising:
>
> a) incubating a composition comprising:
>
> i) the sample, wherein the sample includes poly(A) mRNA;
>
> ii) a poly(dT) or poly(U) nucleic acid molecule; and

iii) tetramethylammonium chloride (TMAC) or tetraethylammonium chloride (TEAC);

under conditions allowing poly(A) mRNA to hybridize with the poly(dT) or poly(U) nucleic acid molecule and inhibiting partial hybridization of the poly(A) mRNA to any rRNA that may be present in the sample; and

b) isolating the poly(dT) or poly(U) nucleic acid molecule and the hybridized poly(A) mRNA;

wherein rRNA carryover is reduced.

Present claim 31 is directed to:

A method for purifying poly(A) mRNA from a sample in a manner that reduces rRNA carryover comprising:

a) incubating the sample with a poly(dT) oligonucleotide connected to a non-reacting structure and a hybridization solution comprising tetramethylammonium under conditions allowing poly(A) mRNA to hybridize with the oligonucleotide;

b) isolating the oligonucleotide with the hybridized poly(A) mRNA away from the sample; and

c) washing the oligonucleotide with a wash solution comprising a salt;

wherein rRNA carryover is reduced.

The currently claimed methods and kits, as described in the specification, are inventive based upon the inventor's discovery that the use of TMAC and/or TEAC in a hybridization solution in the context of purifying poly(A) mRNA results in a greatly reduced rRNA carryover, relative to procedures performed using prior methods.

Previously, contamination by rRNA has caused significant problems for the isolation of poly(A) mRNA, as described in Bantle *et al.* (Analytical Biochemistry, 1976, vol 72, 413-427; Appendix A). However, the interaction between mRNA and rRNA was previously believed to be due to a "nonspecific aggregation" of the rRNA to the solid support (*e.g.*, the cellulose solid

support; see Bantle *et al.*, pages 413, 417, 420, 422-423; Appendix A). In Bantle *et al.*, the use of a "disaggregation step" involving heating the sample in DMSO and/or using multiple passes of the sample through a cellulose column (see pages 420-422) were insufficient to eliminate rRNA contamination. Additionally, the use of these approaches (e.g. multiple passes through a column) can result in significant loss of mRNA.

The previously believed idea that rRNA carryover is solely due to the fact that rRNA is a "sticky molecule" that "nonspecifically aggregates" with other substances (such as the solid cellulose support) is refuted by the inventor's findings that rRNA contamination is due to hybridization of partially-complementary sequences between mRNA and rRNA.

The discovery that rRNA contamination is due to hybridization of partially-complementary sequences between mRNA and rRNA, and the use of TMAC and/or TEAC to prevent this hybridization, thus reducing rRNA carryover, are both novel and non-obvious.

The Action has pointed to no reference suggesting that partial hybridization between mRNA and rRNA is the cause of rRNA carryover. Certainly, the Action points to no reference that suggests that the use of TMAC or TEAC can result in the inhibition of such hybridization and thereby prevent rRNA carryover. "[A] patentable invention may lie in the discovery of the source of a problem even though the remedy may be obvious once the source of the problem is identified. This is *part* of the 'subject matter as a whole,' which should always be considered in determining the obviousness of an invention under 35 U.S.C. § 103." MPEP § 2141.02 (citing *In re Sponnoble*, 405 F.2d 578, 585, 160 U.S.P.Q. 237, 243 (C.C.P.A. 1969)).

Specifically, in Kearney *et al.*, there is no mention of mRNA-rRNA interactions whatsoever. In fact, in Kearney *et al.*, there is neither any mention of mRNA nor any mention of poly(A) mRNA. Thus, it is impossible that Kearney *et al.* discloses the use of TMAC and/or

TEAC to prevent mRNA-rRNA interactions. Kearney *et al.* does not teach a method for inhibiting mRNA-rRNA interactions. Kearney *et al.* does not teach a method for reducing rRNA carryover. In fact, the use of TMAC and TEAC in Kearney *et al.* appears merely to even the binding strength of the two bridging halves of the DNA oligonucleotides, not to inhibit mRNA-rRNA interactions. Indeed, previous to the discovery in the present invention, there is no previous art to demonstrate that rRNA carryover is due to mRNA-rRNA interactions. Kearney *et al.* does not teach all of the elements of the claims of the present invention.

In Aviv *et al.*, the problem of rRNA contamination is also observed when attempting to isolate poly(A) mRNA (see pages 1410-1412). However, not only does Aviv *et al.* not identify the cause of the problem of rRNA contamination, but the authors suggest that a large amount of the peak corresponding to 9S rRNA carryover might actually represent mRNA (p. 1212, paragraph 2). Thus Aviv *et al.* does not even begin to suggest ways to overcome the problem of rRNA carryover. In fact, Aviv *et al.* teaches away from even the identification of the true nature of the problem of rRNA carryover. Neither TMAC nor TEAC are even mentioned in Aviv *et al.* Certainly, neither TMAC nor TEAC are employed to attempt to reduce the problem of rRNA contamination in Aviv *et al.*

Neither Kearney *et al.* nor Aviv *et al.* teach all of the elements of the present claims. Because one skilled in the art would not know the cause of rRNA carryover, one skilled in the art would not be motivated to combine elements in Kearney *et al.* with elements in Aviv *et al.* Additionally, without knowledge of the cause of rRNA carryover, one skilled in the art would not have a reasonable expectation of success in employing TEAC or TMAC in the claimed methods. Therefore, this rejection is overcome.

2. Claims 1-61 Are Allowable Over Kearney *et al.* in View of Aviv *et al.* and Further in View of Jacobs *et al.* and Conlan *et al.*

The Action rejects claims 1-61 under 35 U.S.C. §103(a) as being unpatentable over Kearney *et al.* in view of Aviv *et al.* as applied to claims 1-5, 10-18, 20-28, 30-33, 36-38, 41, 42, 45-50, 53-59, and 61, and further in view of Jacobs *et al.* and Conlan *et al.* The Action relies upon the arguments made with regard to Kearney *et al.* in view of Aviv *et al.* discussed above. Applicant respectfully traverses this rejection for the reasons stated with respect to that combination of references. The combination of Kearney *et al.* and Aviv *et al.* is not the proper basis for a *prima facie* case of obviousness because 1) they do not teach all of the limitations of the claims; 2) there is no motivation or suggestion to combine these references with each other to achieve the claimed invention; and, 3) there is no reasonable expectation that combining these references would achieve the claimed invention.

With respect to the current rejection, the added references of Jacobs *et al.* and Conlan *et al.* do not cure any defects in the *prima facie* case based on Kearney *et al.* and Aviv *et al.* Consequently, the rejection based on these references remains improper. Consequently, Applicant respectfully requests this rejection be withdrawn

3. The Kit Claims 37-38, 41, 42, 45-50, and 61 Are Allowable

Present claims 37 and 60, and their dependent claims, are directed to kits, as follows:

A kit, in a suitable container means, comprising:

a) a poly(dT) oligonucleotide linked to a non-reacting structure; and

b) binding solution comprising TMAC or TEAC.

(Claim 37), and,

A kit, in suitable container means, comprising:

a) a poly(dT) oligonucleotide linked to cellulose;

b) hybridization solution comprising tetramethylammonium (TMAC) in a concentration of between about 1.2 M and about 4 M and Triton X-100 in a concentration of between about 0.03% and about 0.1%;

c) a first wash solution comprising TMAC in a concentration of about 2 M;

d) a second wash solution comprising TMAC in a concentration of about 0.4 M; and

e) elution solution having a total ionic strength of less than 0.01.

(Claim 60).

Independent claims 37 and 60, along with the claims dependent to these claims, are directed to kits. The Action has completely failed to indicate why these composition of matter claims are in any way anticipated or obvious in view of the art. Those with ordinary skill in the art would not be motivated to create said kit because the previous art does not teach the mechanism of rRNA carryover and thus one with ordinary skill in the art would not be motivated to include TMAC or TEAC in a kit containing the claimed components.

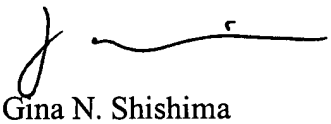
C. Corrected Declaration of Richard C. Conrad

A corrected Declaration of Richard C. Conrad was filed with the response mailed on October 29, 2003. A copy is provided for the examiner's convenience herewith. Certain typographical mistakes accidentally appeared in the previous declaration of Richard C. Conrad regarding the patent application. Specifically, the word "not" was excluded from point #3 in the declaration. Clearly, this represents a typographical error. This error was amended in the corrected declaration previously presented, and no other changes were made to the declaration.

D. Conclusion

In view of the above remarks, Applicant submits that the claims are in condition for allowance. Reconsideration of the application is courteously solicited. The Examiner is invited to contact the undersigned attorney at phone number 512-536-3081 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,



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